

Remarks:

Claims 14-25, 27-30, and 42 remain for consideration in this application with claims 14, 19, 27, and 42 being in independent format.

As an initial matter, Applicant wishes to thank Examiner Betton and his supervisor for their time and consideration during a telephone interview conducted on December 20, 2007. Briefly, Applicant's representatives discussed with the Examiner and his supervisor whether deletion of the phrase "precursors thereof" would overcome the prior art rejections, in addition to overcoming the § 112 issues raised in the Office Action. The Examiner indicated that removing this language would overcome the § 112 issues; however, agreement was not reached with regard to the cited prior art references. Applicant's representatives also discussed with the Examiners whether submitting evidence of the differences between the claimed glycine betaines and the cited prior art compounds would be persuasive in overcoming the cited references. The Examiner indicated that such a declaration may be persuasive.

Turning to the Office Action, claims 14-25, 27-30, and 42 were rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the written description requirement. In particular, the Examiner asserted that the term "precursors" is indefinite. Claims 14, 19, 27-29, and 42 have been amended to eliminate the phrase "precursors thereof." Therefore, this rejection should be overcome.

Applicant notes with appreciation that the Examiner has not raised any prior art rejections against claim 42, which recites that the system is adapted for controlling the release of the active compound for at least 2160 minutes. Now that Applicant has overcome the only rejection raised against this claim (the § 112 rejection) as explained above, claim 42 should be in condition for allowance.

Turning now to the prior art rejections, claims 14-25 and 27-30 were rejected over the combined teachings of U.S. Patent No. 6,287,765 and U.S. Pub. No. 2002/0034757, both to Cubicciotti et al. (hereinafter "Cubicciotti"), in view of U.S. Patent No. 5,928,195 to Malamud et al. (hereinafter "Malamud") and U.S. Patent No. 6,399,785 to Murphy et al. (hereinafter "Murphy"). "To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 U.S.P.Q. 972, 973 (B.P.A.I. 1985)

A determination of obviousness can be problematic because the Examiner, in deciding that a feature is obvious, has the benefit of the applicant's own disclosure as a blueprint or guide, and even a complex solution may seem easy or obvious. In contrast, a person having ordinary skill in the art at the time the invention was made would have no such guide. Furthermore, once an obviousness rejection has been made, the applicant is in the exceedingly difficult position of having to prove a negative proposition (i.e., non-obviousness) in order to overcome this rejection.

For these reasons, the law places upon the Examiner the initial burden of establishing a *prima facie* case of obviousness. See *in re Oteiker*, 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443 (Fed. Cir. 1992). If the Examiner fails to establish the requisite *prima facie* case, the rejection is improper and will be overturned. *In re Rijckaert*, 9 F.3d 1531, 1532, 28 U.S.P.Q.2d 1955 (Fed. Cir. 1993). That is, "[i]f the PTO fails to meet this burden, then the application is entitled to the patent." *In re Glaug*, 283 F.3d 1335, 1338, 62 U.S.P.Q.2d 1151 (Fed. Cir. 2002). Only if the Examiner has satisfied the initial burden of establishing a proper *prima facie* case of obviousness, does the burden shift to the applicant to provide argument or evidence to refute the rejection. See *in re Kumar*, 418 F.3d 1362, 1366, 76 U.S.P.Q.2d 1048 (Fed. Cir. 2005).

In meeting this initial burden, it "is impermissible to pick and choose from the prior art references only the portions of the disclosures that support a given proposition, to the exclusion of other parts necessary to the full appreciation of what the references fairly suggest to one skilled in the art." *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965). Thus, the Examiner must cast his or her mind back to the time of the invention, in order to consider the invention from the perspective of a person having ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field. *See, e.g., W. L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 U.S.P.Q. 303 (Fed. Cir. 1983).

When claims are rejected as obvious in view of two or more references, a holding of obviousness must be based on "an apparent reason to combine the known elements in the fashion claimed." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. ___, 82 U.S.P.Q.2d 1385, 1396 (2007). The determination of obviousness is based, not only on whether a person of ordinary skill in the art would be motivated to combine the references to achieve the claimed invention, but also whether there would have been a reasonable expectation of success in doing so. *PharmaStem Therapeutics, Inc. v. Viacell, Inc.*, 491 F.3d 1342, 1360 (Fed. Cir. 2007) (decided after *KSR*, citing *Medichem, S.A. v. Rolabo, S.L.*, 437 F.3d 1157, 1164 (Fed. Cir. 2006)). Mere conclusory statements cannot sustain an obviousness rejection as there must be "some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *In re Kahn*, 441 F.3d 977, 988, 78 U.S.P.Q.2d 1329 (Fed. Cir. 2006) (emphasis added) (cited with approval in *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. at ___, 82 U.S.P.Q.2d 1385, 1396). *Moreover, if the proposed modification or combination would render the prior art invention unsuitable for its intended purpose, or change its principle of operation, then there is no suggestion or motivation to make such modification or combination.* *In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984) (emphasis added). In addition, references cannot be combined or modified where the prior art teaches away from such combination or modification.

United States v. Adams, 383 U.S. 39, 50-52 (1966) (cited with approval in *KSR*, 550 U.S. at ___, 82 U.S.P.Q.2d 1385, 1395); *See also in re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994). Finally, each and every element of the claims must be taught or suggested in the prior art. M.P.E.P. § 2143.03; *see KSR*, 550 U.S. at ___, 82 U.S.P.Q.2d 1385, 1398-1399 (addressing obviousness based on all elements of claim).

In the Office Action, the Examiner asserted Cubicciotti allegedly "teach[es] the same elements of the claimed invention " but "*does not* teach the use with glycine betaine or any betaine derivative thereof." Office Action, 9/20/2007, p. 2, 6 (emphasis added). To supplement the deficiencies of the Cubicciotti references with regard to the claimed invention, the Examiner cited Malamud as "suggest[ing] the motivation to combine with Cubicciotti et al., because Malamud et al. teach use with a betaine derivative and a glycine derivative in a remotely controlled drug delivery system." *Id.* In addition, the Examiner cited Murphy as "teach[ing] the controlled release of active drug substances in various compartments of the mammal in need of such treatment," and asserted that "Murphy also teaches administration with glycine or derivatives thereof." *Id.* p. 3. The Examiner went on to explain that the claims did not exclude possible derivatives of glycine betaine because of the recited "precursors thereof" in the claims. *Id.*

Applicant respectfully submits that the Examiner has failed to establish a *prima facie* case of obviousness for at least the following reasons. As noted above, claims 14, 19, and 27-29 have been amended to eliminate the phrase "precursors thereof." Thus, as amended, the claims are directed towards controlled release pharmaceutical systems which include an effective amount of a compound selected from the group consisting of "glycine betaine, pharmaceutically acceptable salts thereof, and mixtures thereof." *None* of the cited prior art references teach or suggest glycine betaine, pharmaceutically acceptable salts thereof, and mixtures thereof, as claimed. Rather, Malamud discloses only an "alkyl-*N*-betaine surfactant" such as "alkyl dimethyl glycine." Col 5, ll. 38; 45.

Likewise, Murphy discloses only substituting fullerenes with "*N*-aryl glycines" as precursor compounds, "*N*-linking of benzhydrylamine," and "*N*-tritylglycine." Finally, the Examiner has already acknowledged that there is no teaching of glycine betaine in Cubicciotti. Thus, none of the cited references disclose the instantly claimed glycine betaines, pharmaceutically acceptable salts thereof, and mixtures thereof. Moreover, even if the prior art did teach glycine betaine (and it does not), the Examiner has not identified any "apparent reason" for a person of ordinary skill in the art to combine or modify the references to achieve the presently claimed invention. *KSR*, 550 U.S. ___, 82 U.S.P.Q.2d at 1396.

Applicant further notes that the Examiner keeps using the phrase "betaines and derivatives thereof" in the Office Actions and thus, appears to assert that the compounds disclosed in the prior art are "derivatives" of the claimed glycine betaine. Applicant respectfully submits that this mischaracterizes the prior art in a way that makes the disclosed compounds seem closer to the claimed glycine betaines, pharmaceutically acceptable salts thereof, and mixtures thereof. Even if this characterization were correct, it is well known that a "presumption of obviousness based on a reference disclosing structurally similar compounds may be overcome where there is evidence showing there is no reasonable expectation of similar properties in structurally similar compounds." M.P.E.P. § 2144.09 (citing *In re May*, 574 F.2d 1082, 197 U.S.P.Q. 601 (C.C.P.A. 1978)). Thus, even if the prior art could be said to teach or suggest glycine betaine "derivatives" that may be considered to be structurally similar to the claimed glycine betaines, there would be no reasonable expectation of similar properties in these alleged "derivatives," as explained in detail below.

The Examiner's attention is directed to the attached Declaration under 37 C.F.R. § 1.132 by Dr. Christian Grandfils, Ph.D., Assistant Professor at the University of Liège in Belgium. His *curriculum vitae* is attached as Exhibit A. In the Declaration, Dr. Grandfils explains the structural, chemical, and physio-chemical differences between the disclosed compounds and the claimed

glycine betaine, and attests that because of these differences, a person of ordinary skill in the art *would not* have found the claimed glycine betaines to be obvious or predictable based upon the individual and/or combined teachings of Cubicciotti, Malamud, and/or Murphy.

In paragraph 4, Dr. Grandfils addresses the Cubicciotti references. In more detail, Dr. Grandfils explains that the premise of Cubicciotti is based upon the ability of the molecules used in the disclosed molecular machines to recognize and self-assemble. This requires that the molecules contain at least one hydrophobic segment and at least one hydrophilic segment, sufficiently separated from each other in the molecular structure. In contrast, glycine betaine is a hydrophilic compound only that is soluble in polar solvents such as water. That is, glycine betaine would not have the requisite self-associating capabilities sought after in Cubicciotti. Accordingly, Dr. Grandfils attests that a person skilled in the art would have no reason to modify the system of Cubicciotti to use glycine betaine because this would defeat the purpose of the invention disclosed in Cubicciotti. In addition, Dr. Grandfils explains that because of structural and chemical difference between the claimed glycine betaine and the disclosed compounds, a person skilled in the art would have no reasonable expectation of success in making this suggested modification.

In paragraph 5, Dr. Grandfils addresses the Malamud reference. Dr. Grandfils explains that Malamud is directed towards the delivery of microbicide drugs comprising surfactants with spermicidal, antiviral, antibacterial, and antifungal activities, such as a class of compounds comprising an alkyl-N-betaine surfactant in combination with an oxide selected from the group consisting of alkyl-N, N-dimethyl amine oxide, N-dihydroxyethylamine oxide, acylamino t-amine oxide, and mixtures thereof. According to Dr. Grandfils, the drug's activity is centered on the association of the surfactant with the oxide to form a stable micellar structure in the compound. As shown in Declaration, the structure of glycine betaine does not correspond to the structure of alkyl-N-betaine.

In addition, the differences in structure between the disclosed alkyl-N-betaine and the claimed glycine betaine give rise to fundamentally different and disparate physical and chemical properties, which are neither predictable nor obvious in view of each other. For example, alkyl-N-betaine surfactants contain an alkyl chain, which Dr. Grandfils explains is responsible for generating the surfactant properties with the associated spermicidal, antiviral, antibacterial, and antifungal activities. This assessment is supported by the attached article by Birnie et al., *Antimicrobial Evaluation of N-Alkyl Betaines and N-Alkyl-N,N-Dimethylamine Oxides with Variations in Chain Length*, 44 *ANTIMICROBIAL AGENTS AND CHEMOTHERAPY*, 2514, 2514 (Sept. 2000) (attached as Exhibit B), which discloses that the alkyl chain on the surfactant becomes integrated with, and disrupts the cell membrane function of, the microorganisms. Moreover, the article teaches that longer alkyl chains are preferred over shorter chains, with C₁₆ being the preferred chain length. *Id.* In addition, the article teaches that the ability of the alky-N-betaine to form the micellar structure is critical to its biological activity. *Id.*

In contrast, as noted by Dr. Grandfils in the Declaration, glycine betaine *does not* exhibit microbicidal properties. Moreover, as noted by Applicant in the previous response, glycine betaine actually provides a favorable environment for microorganisms: (1) it has protective effects on spermatozoa; (2) it favors bacterial growth, and bacteria avidly uptake glycine betaine to protect themselves; and (3) it favors fungal growth and the development of yeast. (See Exhibits E-K that were filed with the June 20, 2007 Amendment). This makes sense because, unlike the surfactants disclosed in Malamud, there is *no alkyl chain* in glycine betaine to interfere with the microorganism's cell membrane and generate the associated spermicidal, antiviral, antibacterial, and antifungal activities. Conversely, based upon the structural and chemical differences between the claimed glycine betaine and the disclosed compounds, Dr. Grandfils further attests that a person skilled in the art would have no reasonable expectation that the alkyl-N-betaine surfactants disclosed in

Malamud would be capable of generating the therapeutic properties of the claimed glycine betaines to treat thrombosis.

In addition, as Dr. Grandfils avers, there would also be no scientific rationale to modify the surfactants disclosed in Malamud to remove the alkyl chain and replace it with a methyl group to arrive at the glycine betaine structure. This is especially true in light of Birnie et al. above, which teaches away from this modification by teaching that *longer* alkyl chains are preferred as they demonstrate better microbial activity. That is, removing the alkyl chain from the betaine surfactant in Malamud would defeat the spermicidal, antiviral, antibacterial, and antifungal activities of the surfactant and render the drug unsuitable for the intended microbicide purposes disclosed in Malamud. This modification would also interfere with the surfactant's interaction with the oxide and inhibit the formation of the micellar structure necessary to create a stable microbicide compound. Thus, because the proposed modification or combination would render the invention of Malamud unsuitable for its intended purpose, or change its principle of operation, there can be no suggestion or motivation to make such modification or combination. *In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984).

Finally, Applicant asserts that Malamud is non-analogous art, and it is improper to use it as the basis of an obviousness rejection against the present application. One inquiry to be made in rendering an obviousness determination, is to determine the scope and content of the prior art. A determination of the scope and content of the prior art involves distinguishing analogous art from non-analogous art. *See In re Clay*, 966 F.2d 656, 658, 23 U.S.P.Q.2d 1058 (Fed. Cir. 1992). Only analogous art should be used when making an obviousness determination. To be considered analogous art, a reference must satisfy one of two criteria. *Id.* at 659–59. First, a reference is considered analogous if it is within the same field of endeavor as the claimed invention, regardless of the problem addressed. *Id.* Alternatively, even if a reference is not within the inventor's field of

endeavor, the reference may still be analogous if it is reasonably pertinent to the particular problem with which the inventor is involved. *KSR*, 550 U.S. ___, 82 U.S.P.Q.2d at 1397. That is, a reference is analogous art if "it is one which, because of the matter with which it deals, logically would have commended itself to [the] inventor's attention in considering his problem." M.P.E.P. § 2141.01(a).

When considering the first criterion, it is clear that Malamud is not within the same field of endeavor as the present invention. Rather, Malamud is concerned with the field of prophylactics and microbicides for prevention of pregnancy and "protection from or treat[ment] of sexually transmitted diseases (STDs), including herpes, syphilis, gonorrhea, chlamydia and HIV." Col. 1, ll. 35-43. The prophylactics and microbicides are delivered via a remotely controlled delivery device, specifically a "vaginal ring or other shape housing." Col. 1, ll. 35-37. To this end, Malamud discloses using surfactants, such as alkyl-N-betaine, that have microbicidal, spermicidal, and antifungal properties. Col. 5, ll. 34-38. In contrast, the present invention relates to the field of treating thrombo-embolic and haemostatic diseases of arterial or venous origin. Applicant submits that a person of ordinary skill in the art of treating arterial/circulatory diseases would not have any incentive or motivation to look to the field of STDs, prophylactics, microbicides, and vaginal rings as they are wholly unrelated fields of endeavor.

The next criterion to consider in determining whether Malamud is analogous art is whether that reference is reasonably pertinent to the problem that the inventors of the present claims were addressing. This criterion is also not met by Malamud. The present invention solves problems of treating and preventing arterial and circulatory thrombosis without the risk of hemorrhage or allergy that commonly result from prior art compounds. Malamud is not at all pertinent to this problem. Rather, Malamud is concerned with providing "discreet, safe and effective" intravaginal drug delivery devices that do not suffer from the drawbacks of prior art systems which use gas pressure to force a dose of the drug into the body cavity. Col. 1, ll. 15-32, 45. Thus, Malamud addresses a

completely different problem than the one faced by the inventors of the present invention. It cannot be said that Malamud "logically would have commended itself to [the] inventor's attention in considering his problem." M.P.E.P. § 2141.01(a). Thus, because neither of the relevant criteria are met by this reference, it is respectfully submitted that Malamud is non-analogous art and should not be used as a basis for an obviousness rejection against the present application.

Turning back to the Grandfils Declaration, in paragraph 7 Dr. Grandfils addresses the Murphy reference. In more detail, Dr. Grandfils explains that Murphy is concerned with substituted fullerenes, which have completely different chemical structures compared to the recited glycine betaines. In particular, fullerenes are comprised of carbon atoms and form hollow spheres, ellipsoids, or tube shapes. Reacting a fullerene with a glycine derivative, such as N-aryl glycine, produces an R-substituted fullerene with a structure that is nowhere near that of glycine betaine, as illustrated in the Declaration. Moreover, as seen in Col. 24, ll. 10-40 of Murphy, even the intermediate product is still totally different from glycine betaine. In addition, Dr. Grandfils avers that the properties of fullerenes are well known in the art, and the disclosed substituted fullerenes would have properties totally different from glycine betaine in terms of chemical structure (as can be seen in the Declaration), molecular weight, and solubility. Finally, there is actually no mention at all of betaines in Murphy. Thus, Dr. Grandfils attests that a person of ordinary skill in the art would not find the claimed glycine betaines to be obvious or predictable based upon information provided in Murphy.

According to Dr. Grandfils, there is simply no scientific rationale in Cubicciotti, Murphy, or Malamud, taken individually or combined, which could explain or provide a reasonable expectation of success in achieving the surprising and unexpected results of the invention claimed in the present application, based upon the information provided in these references and the knowledge available to persons of ordinary skill in the art at the time of the invention. Therefore,


as the claimed invention would not be obvious to a person skilled in the art at the time of the invention, independent claims 14, 19, 27, and 42 are patentable over the art of record, and it is respectfully requested that the rejections against these claims be withdrawn.

In addition, while dependent claims 15-18, 20-25, and 28-30 recite additional patentable features, these claims should also be in condition for allowance, as depending from patentable independent claims. *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988).

As a final matter, Applicant notes that the corresponding international application PCT/BE00/00021 has been granted in the European Patent Office as EP 1156796 on November 30, 2005, and corresponding international application PCT/BE02/00013 has been granted in the European Patent Office as EP 1408949 on November 22, 2007.

In view of the foregoing, it is believed that no further issues exist with respect to this application. The Applicant respectfully requests a Notice of Allowance. Any additional fees due in conjunction with this amendment should be applied against our Deposit Account No. 19-0522.

Respectfully submitted,

By 
Tracy L. Bornman, Reg. No. 42,347
HOVEY WILLIAMS LLP
10801 Mastin Blvd., Suite 1000
84 Corporate Woods
Overland Park, Kansas 66210
ATTORNEYS FOR APPLICANT(S)

Antimicrobial Evaluation of *N*-Alkyl Betaines and *N*-Alkyl-*N,N*-Dimethylamine Oxides with Variations in Chain Length

CHRISTINE R. BIRNIE,¹ DANIEL MALAMUD,^{2,3} AND ROGER L. SCHNAARE^{1*}

Department of Pharmaceutical Sciences, Philadelphia College of Pharmacy, University of the Sciences in Philadelphia,¹ and Department of Biochemistry, School of Dental Medicine, University of Pennsylvania,² and Biosyn, Inc.,³ Philadelphia, Pennsylvania 19104

Received 13 January 2000/Returned for modification 13 March 2000/Accepted 5 June 2000

Alkyl betaines and alkyl dimethylamine oxides have been shown to have pronounced antimicrobial activity when used individually or in combination. Although several studies have been conducted with these compounds in combinations, only equimolar concentrations of the C_{12}/C_{12} and C_{16}/C_{14} chain lengths for the betaine and the amine oxide, respectively, have been investigated. This study investigates the antimicrobial activity of a wide range of chain lengths (C_8 to C_{18}) for both the betaine and amine oxide and attempts to correlate their micelle-forming capabilities with their biological activity. A broth microdilution method was used to determine the MICs of these compounds singly and in various molar ratio combinations. Activity against both *Staphylococcus aureus* and *Escherichia coli* was investigated. Antimicrobial activity was found to increase with increasing chain length for both homologous series up to a point, exhibiting a cutoff effect at chain lengths of approximately 16 for betaine and 14 for amine oxide. Additionally, the C_{18} oleyl derivative of both compounds exhibited activity in the same range as the peak alkyl compounds. Critical micelle concentrations were correlated with MICs, inferring that micellar activity may contribute to the cutoff effect in biological activity.

As more resistant organisms continue to emerge in society, the identification of additional antimicrobial agents becomes increasingly more important. Compounds such as surfactants are an area to be investigated. Betaines and amine oxides, two types of amphoteric surfactants, have been shown to exhibit antimicrobial activity against a variety of microorganisms (7, 16, 18, 25). Although each of these compounds has shown pronounced activity alone, they have also been used in combination to exhibit a synergistic effect (6).

An equimolar mixture of *N*-alkyl betaine and *N*-alkyl-*N,N*-dimethylamine oxide was patented in 1978 in a compound called C31G (17). With chain lengths ranging from C_8 to C_{18} and buffered in a citrate buffer, C31G was first shown to have pronounced wound healing and deodorizing effects, as well as antimicrobial sensitivity. Further studies showed C31G has exhibited pronounced activity not only against bacteria, but also against yeasts, fungi, sperm, and enveloped viruses (4, 6, 14, 23). Although several studies have been published about this compound in reference to the extent of antimicrobial activity, little work has been conducted with any other chain lengths besides the following two chain-length combinations: (i) C_{12} betaine- C_{12} amine oxide and (ii) C_{16} betaine- C_{14} amine oxide. Additionally, only an equimolar ratio of the two compounds has been investigated.

The structures of these two components are shown in Fig. 1. The variation in length of the long hydrocarbon tail is thought to influence the extent of antimicrobial activity. Like most other surfactants, they are believed to be membrane perturbants, disrupting the cell membrane of the microorganism (26). It is believed that interaction with the surface of the microorganism is a function of the polar head groups of the

betaine, amine oxide, or mixture of these molecules and that the hydrocarbon tail subsequently becomes integrated with the lipid bilayer of the cell membrane. This integration causes a disruption in the membrane and inevitably causes leakage of the cell contents. The length of the alkyl chain of the surfactants is thought to contribute to the extent of this membrane disruption, because the higher chain lengths may be incorporated into the lipid bilayers of the plasma membrane. The increased hydrophobic effect of these longer chain tails may aid in this disruption (16).

In an effort to find an optimal combination of betaine and amine oxide, our study evaluated the extent of antimicrobial activity of a homologous series of betaines, amine oxides, and combinations of these compounds. Being surfactants, their micelle-forming capability is also correlated with their biological activity.

MATERIALS AND METHODS

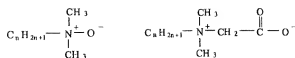
Betaine and amine oxides. Compounds ranging in chain length from 8 to 18 carbons for *N*-alkyl betaine and *N*-alkyl-*N,N*-dimethylamine oxide were obtained from two manufacturers, McIntyre Group, Ltd. (University Park, Ill.), and Stephan Co. (Northfield, Ill.). Although not all chain lengths were available, a representative group of samples was acquired. In addition to the alkyl straight chains, oleyl derivatives [$C_{18}(9)$] of both betaine and amine oxide were also obtained for analysis. Table 1 shows the compounds tested and their manufacturers.

Microorganisms. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were obtained from the American Type Culture Collection (ATCC) and used as representative gram-negative and gram-positive organisms. Both microorganisms were maintained in Mueller-Hinton agar and broth, buffered to pH 4.8 for *E. coli* and maintained at pH 7.3 for *S. aureus*. Citric acid buffer (2.5 mM) at pH 5.5 and 7.3 was used as a diluting solution as needed.

Microorganisms were stored in freezer vials at -10°C under conditions recommended by the ATCC. As needed, samples were thawed, warmed to 37°C , and streaked by dilution on agar petri plates for isolation. Inoculated plates were placed in a humidified incubator at 37°C for 24 h, during which time colonies formed. With a sterile loop, colonies were picked and dispersed in broth. After 24 h of incubation, the concentration of microorganisms was adjusted to a turbidity equal to that of a 0.5 McFarland standard, adjusted by diluting the overnight culture to a concentration equivalent to 80% transmittance (625 nm).

* Corresponding author. Mailing address: Department of Pharmaceutical Sciences, Philadelphia College of Pharmacy, University of the Sciences in Philadelphia, Philadelphia, PA 19104. Phone: (215) 596-8942. Fax: (215) 895-1100. E-mail: r.schnaare@usp.edu.





N-Alkyl- N,N-dimethyl-amine oxide

N-Alkyl-betaine

FIG. 1. Chemical structures of N-alkyl-N,N-dimethylamine oxide and N-alkyl betaine.

This standardized suspension has been shown to contain approximately 10^6 CFU/ml (19).

Antimicrobial evaluation. A microdilution plate method was used according to National Committee for Clinical Laboratory Standards methods with Mueller-Hinton broth (19). In 96-well dishes, concentrations of N-alkyl betaine and N-alkyl-N,N-dimethylamine oxide ranged from 10^4 to $1 \mu\text{M}$, with the final well column containing no active sample solution. The standardized microorganism suspension was added to each well, and plates were incubated in a 37°C humidified incubator for 24 h before evaluation. The MICs were determined based on visual observation of turbid and nonturbid wells. Samples were run in duplicate.

CMCs. Selected critical micelle concentrations (CMCs) were determined by using measured surface tension values as a function of concentration. Surface tension measurements at 25°C were determined by the Wilhelmy plate method on a Rosano surface tensiometer. The CMCs were determined by plotting the surface tension against the log of the concentration. The CMC is noted as the sharp change in decreasing surface tension as the concentration of surface active agent is increased.

RESULTS

Betaine. The antimicrobial activity of a homologous series of N-alkyl betaines was evaluated against *S. aureus* and *E. coli*. Table 2 shows the MICs of these compounds. Antimicrobial activity was very poor at lower chain lengths, with MICs of C_8 betaine of $2.3 \times 10^4 \mu\text{M}$ for *S. aureus* and $1.2 \times 10^4 \mu\text{M}$ for *E. coli*. The MICs of the betaine series decreased with increasing chain length, plateauing at the higher chain lengths—around C_{16} for both microorganisms. The C_{16} compound exhibited some of the best activity, with MICs of 61 and $120 \mu\text{M}$ for *S. aureus* and *E. coli*, respectively.

Amine oxide. Table 3 shows the MIC results for the series of homologous N-alkyl-N,N-dimethylamine oxides. Like the betaine series, the amine oxide series followed a similar trend of increased activity with increased chain length. Again exhibiting very poor activity at the low chain lengths, the MICs of the C_8 amine oxide were 2.9×10^4 and $3.6 \times 10^4 \mu\text{M}$ for *S. aureus* and *E. coli*, respectively. The activity also increased with chain length, up to approximately C_{14} to C_{16} , and then tailed off at the higher chain lengths. For the amine oxide series, activity peaked at a chain length of C_{14} against *E. coli*, at a MIC of $31 \mu\text{M}$, and plateaued at C_{14} to C_{16} against *S. aureus*, at a MIC of $62 \mu\text{M}$. In addition, the C_{18} oleyl compound showed excellent activity against both microorganisms, matching the alkyl chain's peak activity. Being unsaturated in chemical structure,

TABLE 1. N-Alkyl betaine and N-alkyl-N,N-dimethylamine oxide derivatives available from manufacturers

Chain length	Manufacturer	
	Betaine	Amine oxide
C_8	McIntyre	McIntyre
C_{10}	McIntyre	McIntyre
C_{12}	McIntyre	McIntyre or Stepan
C_{14}		Stepan
C_{16}	McIntyre	Stepan
C_{18}		Stepan
C_{18} (oleyl)	McIntyre	McIntyre

TABLE 2. MICs of a homologous series of N-alkyl betaines for both *S. aureus* and *E. coli*

Compound	MIC (μM)	
	<i>S. aureus</i>	<i>E. coli</i>
C_8 betaine	2.3×10^4	1.2×10^4
C_{12} betaine	150	290
C_{16} betaine	61	120
$\text{C}_{18(\text{ol})}$ betaine	110	230

the oleyl compounds do not exhibit the poor solubility problems usually associated with the C_{18} stearyl compounds.

CMCs. The CMCs for N-hexadecyl betaine and N-tetra-decyl-N,N-dimethylamine oxide were determined by using surface tension measurements as a function of concentration. The remaining CMCs have been collected from the literature and are shown in Table 4. It is well known in the literature that a linear relationship exists between chain length and CMC (22). For a homologous series, the following equation has been used: $\log \text{CMC} = k_1 N + k_2$, where N is equal to the number of carbon atoms in the alkyl chain and k_1 and k_2 are constants. A linear relationship was determined for the betaine series in this study, yielding the equation $\log \text{CMC} = -0.447 N + 4.10$ ($R^2 = 0.995$). The amine oxide series also followed a linear trend, yielding the equation $\log \text{CMC} = -0.438 N + 3.89$ ($R^2 = 0.998$). Even with compiled CMCs, determined under different conditions, the linear correlation is still very good.

DISCUSSION

Cutoff effect. Unlike the log-linear relationship between increasing alkyl chain length and CMC for an entire homologous series, both the betaine and amine oxide series showed this linear relationship with the antimicrobial activity only at the lower chain lengths. Figures 2 and 3 show the relationship of concentration versus alkyl chain length, comparing both the MIC and the CMC. The MICs of both the betaine and the amine oxide series for both microorganisms exhibited a plateauing or parabolic effect with increasing alkyl chain lengths, occurring at chain lengths of approximately 14 to 16. This phenomenon is consistent with current literature regarding biological activity, with numerous studies documenting this type of response with homologous series of long-chain amphiphilic molecules (13, 22, 24).

Ferguson was one of the first to document this type of effect in 1939 (8) when compiling a combination of studies relating to homologous series of compounds. He referred to this change in biological activity as exhibiting a "cutoff effect" at higher chain lengths.

Several theories have been postulated as to why this cutoff

TABLE 3. MICs of a homologous series of N-alkyl-N,N-dimethylamine oxides for both *S. aureus* and *E. coli*

Compound	MIC (μM)	
	<i>S. aureus</i>	<i>E. coli</i>
C_8 amine oxide	2.9×10^4	3.6×10^4
C_{10} amine oxide	3.1×10^3	340
C_{12} amine oxide	340	87
C_{14} amine oxide	62	31
C_{16} amine oxide	56	56
C_{18} amine oxide	102	390
$\text{C}_{18(\text{ol})}$ amine oxide	51	25

TABLE 4. CMCs of *N*-alkyl betaine and *N*-alkyl-*N,N*-dimethylamine oxide as a function of alkyl chain length

Alkyl chain length	CMC (μM) ^a	
	Betaine	Amine oxide
8	1.86×10^3 (1)	1.50×10^3 (2)
10	1.80×10^4 (1)	1.5×10^4 (21)
12	1.84×10^3 (1)	1.70×10^3 (10)
14	186 (1)	268
16	20.1	24.9 (10)
18	4.78 (9)	

^a Reference numbers are given in parentheses.

effect occurs. Janoff and Pringle (12, 20) have associated this cutoff with a limit in solubility. They proposed that as the alkyl chain increases, lipid solubility increases at a rate faster than the change in partition coefficient (lipid/aqueous). At these higher chain lengths, partitioning is limited, making the concentration at the site of action insufficient to have a significant effect on the membrane of the cell wall (12, 20). Other accounts attribute this to a decrease in perturbation of the membrane at higher chain lengths, proposing that the longer alkyl chain molecules better mimic molecules in the lipid bilayer, causing less of a disruption in the membrane (7).

Although numerous theories exist, it is likely that in the case of the betaine and amine oxide series, the cutoff in biological activity is due to the micellar action of these compounds. Ross and coworkers first suggested this theory, investigating alkylbenzyltrimethylammonium chlorides of various chain lengths (22). As surfactants increase in chain length, their tendency toward micelle formation is greater, noted by the lower CMCs at higher chain lengths. This tendency to form micelles becomes greater than the tendency to move toward the interface (the membrane), and thus the concentration at the site of action becomes decreased. Also, as the size of the diffusing species increases from the size of a monomer to that of micelles, their diffusibility and permeation abilities will decrease, affecting their action on the microbial cell wall.

Comparing the CMC and the MIC in Fig. 2 and 3, the linear CMC line intersects the MIC line at the cutoff point for both the betaine and amine oxide series with both microorganisms investigated. At chain lengths below the cutoff point, the MICs of both compounds are below the CMC line, implying that the monomeric species of these compounds are responsible for an antimicrobial response. Although some micelles may be present at concentrations below the CMC, it is not likely they are significant enough to produce an effect. In the case of the amine oxide series, the MICs for *E. coli* are up to 2 orders of

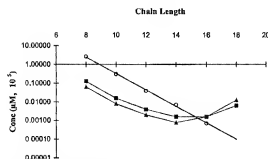


FIG. 3. Correlation of MIC with CMC for a homologous series of *N*-alkyl-*N,N*-dimethylamine oxides. ○, CMC; ■, MIC for *S. aureus*; ▲, MIC for *E. coli*.

magnitude lower than the CMC. At these concentrations, it is not likely that the presence of micelles is significant.

At chain lengths above the cutoff point, the MIC is not reached until well above the CMC. Possessing a much lower CMC than the short-chain homologues, fewer monomers will be present at these concentrations, apparently less than are needed to produce a significant biologic effect. Increased overall concentrations are needed to obtain the desired bactericidal effects.

Effects of differences in bacterial strains. It is also to be noted that both the betaine and amine oxide series show similar trends in activity for both *S. aureus* and *E. coli*, with both organisms exhibiting a cutoff effect in approximately the same location. These results are advantageous in a compound selection process, in which only one chain length that exhibits the best broad-spectrum activity can be selected for further development.

Although the similar effect on both the gram-positive and gram-negative organisms is preferred, it is not always the case with different strains of microorganisms. Ferguson noted that the most resistant organisms will often exhibit a cutoff effect much lower in alkyl chain length, while Lien and Hansch more specifically concluded that gram-positive organisms preferred a more lipophilic molecule than the gram-negative one (15). This has been attributed to the cell wall difference between bacterial types and strains. *E. coli*, a gram-negative rod, exhibits a more complex cell wall than gram-positive organisms such as *S. aureus* (5). Although both gram-positive and -negative organisms have a similar cytoplasmic membrane inside the outer wall, containing both phospholipids and membrane proteins, the outer walls are very different. Gram-positive bacteria have a very simple cell wall, consisting mainly of a mesh-like structure, while the gram-negative bacterial cell walls contain a layer of peptidoglycan between an outer membrane and the cytoplasmic membrane. This outer membrane contains lipopolysaccharides which are cross-bridged by divalent cations, believed to aid in the stabilization of the outer membrane and also make the membrane more impermeable to lipophilic molecules (3).

Oleyl compounds. In addition to the cutoff peak of the homologous series, the oleyl compounds showed very good antimicrobial activity. The compounds are unsaturated and exhibit a much greater aqueous solubility than that of the other high chain lengths, particularly the C_{18} stearyl. They likely possess the right lipophilic/hydrophilic balance to allow the molecule to adequately disrupt the cell wall of the microorganism. The CMC of the 9-octadecyl-*N,N*-dimethylamine oxide has been shown to be 128 μM (11), which is comparable to the CMC of the most antimicrobially active alkyl compounds. Additionally, the CMC of this derivative is greater than the

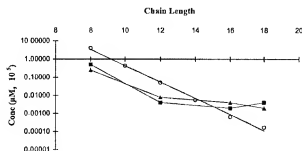


FIG. 2. Correlation of MIC with CMC for a homologous series of *N*-alkyl betaines. ○, CMC; ■, MIC for *S. aureus*; ▲, MIC for *E. coli*.

MIC, further supporting the contention that the antimicrobial aspects of these compounds are primarily due to that of the monomer.

Conclusions. Overall, by comparing the MIC and CMC of the betaine and amine oxide series, this study has provided a better understanding of the relationship between the biological activities of these compounds correlated with their micelle-forming capabilities. It was shown that the majority of compounds provide excellent antimicrobial activity in their monomeric forms, but at chain lengths above the cutoff point, compounds must be in both a micellar form and monomeric form to exhibit a similar antimicrobial effect. Additionally, a range of chain lengths that exhibited some of the best antimicrobial activity was identified for both compounds. For both the betaine and amine oxide, compounds in the range of C_{14} to C_{16} were shown to be among the most effective of the alkyl compounds in addition to the unsaturated C_{18} olefins.

ACKNOWLEDGMENT

This work was supported in part by the Biosyn Graduate Research Fellowship.

REFERENCES

- Beckett, A., and R. Woodward. 1963. Surface-active betaines: N-alkyl-N-dimethylglycines and their critical micelle concentrations. *J. Pharm. Pharmacol.* 15:422-431.
- Benjamin, L. 1964. Calorimetric studies of the micellization of dimethyl-n-alkylamine oxides. *J. Phys. Chem.* 68:3575-3581.
- Brooks, G., J. Butel, and L. Ornston. 1991. Jawetz, Melnick and Adelberg's medical microbiology, 19th ed., p. 15-20. Appleton and Lange, Norwalk, Conn.
- Call, S., N. Yalug, M. Sumnu, A. Ayhan, and A. Hincal. 1992. A non-antibiotic antimicrobial mixture (C31G): evaluation of the antimicrobial efficiency of C31G on vaginal culture. *Bull. Chim. Farm.* 131:335-338.
- Campbell, N. 1993. *Biology*, 3rd ed., p. 517. Benjamin Cummings Publishing, Redwood City, Calif.
- Cornier, A.-M., M. M. Doku, S. L. Yankell, and D. Malamud. 1988. C31G, a new agent for oral use with potent antimicrobial and antifluorescence properties. *Antimicrob. Agents Chemother.* 32:350-353.
- Devinsky, F., A. Kopecka-Leitmanova, F. Sersen, and P. Balgavy. 1990. Cut-off effect in antimicrobial activity and in membrane perturbation efficiency of the homologous series of N,N-dimethylalkylamine oxides. *J. Pharm. Pharmacol.* 42:790-794.
- Ferguson, J. 1939. The uses of chemical potentials as indices of toxicity. *Proc. R. Soc. Lond. B* 127:387-404.
- Harrison, D., R. Szule, and M. Fisch. 1998. Solution behavior of the zwitterionic surfactant octadecyldimethylbetaine. *J. Phys. Chem.* 102:6487-6492.
- Hollman, H. 1990. Correlation between surface and interfacial tensions with micellar structures and properties of surfactant solutions. *Prog. Colloid Polym. Sci.* 83:16-28.
- Imae, T., H. Araki, and S. Ikeda. 1986. The absorption spectra and the micelle species of dimethyloleamine oxide in aqueous solutions. *Colloids Surf.* 17:221-228.
- Janoff, A., M. Pringle, and K. Miller. 1981. Correlation of general anesthetic potency with solubility in membranes. *Biochim. Biophys. Acta* 649:125-128.
- Kanazawa, A., T. Ikeda, and T. Endo. 1994. Synthesis and antimicrobial activity of dimethyl- and trimethyl-substituted phosphonium salts with alkyl chains of various lengths. *Antimicrob. Agents Chemother.* 38:945-952.
- Krebs, F., S. Miller, D. Malamud, M. Howett, and B. Wigdahl. 1999. Inactivation of human immunodeficiency virus type 1 by nonoxonyl-9, C31G, or an alkyl sulfate, sodium dodecyl sulfate. *Antivir. Res.* 43:157-173.
- Lien, E., C. Hansch, and S. Anderson. 1968. Structure activity correlations for antimicrobial agents on gram-positive and gram-negative bacteria. *J. Med. Chem.* 11:430-441.
- Lindstedt, M., S. Allemark, R. A. Thompson, and L. Edebo. 1990. Antimicrobial activity of betaine esters, quaternary ammonium amphiphiles which spontaneously hydrolyze into nontoxic components. *Antimicrob. Agents Chemother.* 34:1949-1954.
- Michaels, E. B. August 1978. U.S. patent 4,107,328.
- Mynarczyk, D., V. Cnpkova, F. Devinsky, and I. Lacko. 1978. Antimicrobial efficiency of saturated heterocyclic amine oxides. *Folia Microbiol.* 23:493-495.
- National Committee for Clinical Laboratory Standards. 1991. NCCLS document M7-A2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- Pringle, M., K. Brown, and K. Miller. 1981. Can the lipid theories of anesthesia account for the cut off in anesthetic potency in homologous series of alcohols? *Mol. Pharmacol.* 19:49-55.
- Rathman, J., and S. Christian. 1990. Determination of surfactant activities in micellar solutions of dimethyldodecylamine oxide. *Langmuir* 6:391-395.
- Ross, S., C. Kwartler, and J. Bailey. 1953. Colloidal association and biological activity of some related quaternary ammonium salts. *J. Colloid Sci.* 8:385-401.
- Thompson, K., D. Malamud, and B. Storey. 1996. Assessment of the antimicrobial agent C31G as a sporicide: comparison with nonoxonyl-9. *Contraception* 53:313-318.
- Tomlinson, K., M. Brown, and S. Davis. 1977. Effect of colloidal association on the measured activity of alkylbenzyltrimethylammonium chlorides against *Pseudomonas aeruginosa*. *J. Med. Chem.* 20:1277-1282.
- Tsabone, K., N. Uchida, and Y. Ito. 1991. Relation between structure and antimicrobial activity of 2-(N,N,N-trialkylammonio)alkyl hydrogen phosphates. *J. Pharm. Sci.* 80:441-444.
- Wyrick, P. B., S. T. Knight, D. G. Gerbig, Jr., J. E. Raulston, C. H. Davis, T. R. Paul, and D. Malamud. 1997. The microbial agent C31G inhibits *Chlamydia trachomatis* infectivity in vitro. *Antimicrob. Agents Chemother.* 41:1335-1344.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:

MESSADEK, Jallel

Serial No.: 10/635,048

Filed: August 4, 2003

GLYCINE BETAINE AND ITS USE

Docket No.: 31927-CIP2

Confirmation No.: 6961

Group Art Unit No.: 1614

Customer No.: 23589

Examiner: Betton, Timothy E.

Commissioner for Patents
Mail Stop Amendment
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

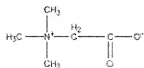
DECLARATION UNDER 37 C.F.R. § 1.132

I, Dr. Christian Grandfils, do declare and state as follows:

1. I am a citizen of Belgium and reside in Liège, Belgium. I am over the age of eighteen, under no disability, fluent in English and understand the following statements made regarding the above-referenced patent application and the prior art references cited by the Examiner. I have a Ph.D. in Biomedical and Experimental Sciences from the University of Liège, Belgium. I am currently an Assistant Professor and a member of the medicine faculty at the University of Liège. I am also the Director of the Interfaculty Center for Biomaterials (CEIB) at the University. My research over the past 25 years has focused on tissue engineering, biochemistry, drug delivery systems, optimization of diagnostic systems, and *in vitro* biocompatibility testing of biomaterials. My *curriculum vitae* is attached as Exhibit A.

2. I understand that the Examiner has rejected the claims as being obvious in view of three U.S. Patents and one published U.S. Patent Application: U.S. Patent No. 5,928,195 to Malamud et al. (hereinafter "Malamud"); U.S. Patent No. 6,399,785 to Murphy et al. (hereinafter "Murphy"); and U.S. Patent No. 6,287,765 and U.S. Patent Pub. No. 2002/034757 to Cubicciotti et al. (hereinafter "Cubicciotti"). I have reviewed these references along with the instant application and have compared the claimed invention to the disclosures of the cited prior art. I have been asked to provide this declaration to attest to the state of the art prior to, and on, the filing date of this application, including the cited references, and to attest to the nonobviousness of the claimed invention.

3. The present invention provides pharmaceutical uses of betaines, and specifically glycine betaine, such as for the treatment of thrombosis and/or blood disorders. Independent claims 14, 19, 27, 42 are directed towards controlled release pharmaceutical systems which include an effective amount of a compound selected from the group consisting of glycine betaine, pharmaceutically acceptable salts thereof, and mixtures thereof. Each system delivers or releases the glycine betaine in a time controlled manner. The structure of glycine betaine is:



The significance of the present invention is based on the fact that the use of glycine betaine does not result in any risk of hemorrhage or allergy, as opposed to the molecules and treatments currently used. This is a critical and unpredictable property of the controlled release pharmaceutical systems. With the support of various experimental observations carried out either *in vitro* or *in vivo* on animals and on human beings, Mr. Jallal Messadek, the inventor named in the present application,

has demonstrated the unique therapeutic advantages of these betaines, in particular for the treatment, prevention, and/or stabilization of problems relating to blood circulation, in particular to the blood microcirculation. The present invention is also advantageous as providing several possibilities to have controlled release preparations and/or devices comprising glycine betaine in order to achieve a pharmacological active concentration of glycine betaine in the bloodstream.

4. After carefully reading the Cubicciotti, Malamud, and Murphy references, according to my experiences in chemistry for the optimization of drug delivery systems and according to the state of the art, a person skilled in the art at the time of the instant invention would not have been able to deduce or derive the claimed controlled release glycine betaine, based upon the teachings and information in these references. None of the references cited above disclose the potency and performance of glycine betaine with respect to venous and/or arterial thrombosis, or its anti-aggregant and anticoagulant potency. Furthermore, these properties are not something that would be achievable with the prior art by simply using routine experimentation. That is, when taken either individually or combined, nothing in these references could support or explain the features claimed in the above-referenced application, as the present invention yields surprising and unexpected results over the prior art, as explained in more detail below.

CUBICCIOTTI ET AL. (U.S. Patent No. 6,287,765 and U.S. Patent Pub. No. 2002/0034757)

5. The aim and technical approach of Cubicciotti is directed towards methods for detecting and identifying single molecules, based upon the self-association of compounds in multimolecular devices and drug delivery systems prepared from synthetic heteropolymers, heteropolymeric discrete structures, multivalent heteropolymeric hybrid structures, etc. To a person skilled in the art of drug delivery systems, the teachings of Cubicciotti are completely different from,

and unrelated to, the objectives and compounds disclosed in the present application.

Recognition and self-assembly are two critical properties of the compounds sought in Cubicciotti. Based upon the teachings of Cubicciotti, it would not have been obvious to a person skilled in the art at the time of the invention to modify the teachings of Cubicciotti to use glycine betaine as claimed. For self-association, it is well established that an amphiphilic molecule should contain at least two segments with respective hydrophobic and hydrophilic properties, which are sufficiently separated to provide the requisite self-associating ability. These amphiphilic properties would not be expected based upon the structure of glycine betaine, and none have been reported in the literature. This makes sense because glycine betaine is a hydrophilic compound only soluble in polar solvents such as water. Therefore, a person skilled in the art would not be motivated to modify any of the compounds disclosed in Cubicciotti to arrive at the claimed glycine betaines because this would defeat the self-associating ability of the molecules, which is critical to the invention of Cubicciotti.

In addition, because of the fundamental differences in the chemistry of glycine betaine and the compound disclosed in Cubicciotti, a person skilled in the art would have no reasonable expectation of success in substituting glycine betaine in the preparation of any of the molecular machine systems described in Cubicciotti:

- synthetic heteropolymers which comprise a first synthetic defined sequence segment capable of specifically recognizing and covalently attaching a first selected monoligonucleotide molecule and a second defined sequence segment attached to the first synthetic defined sequence segment with the proviso that the second defined sequence segment is not a fixed, unconjugated primer-annealing sequence;
- heteropolymeric discrete structures which comprise a synthetic aptamer and a defined sequence segment attached to the synthetic aptamer;
- a molecular adsorbents which comprise a solid phase and a multivalent template having a first specific recognition element specifically attached via the first specific recognition element to the solid phase;
- multimolecular adherents which comprise a specific recognition element and a first selected molecule attached to the specific recognition element;

- multimolecular adhesives which comprise at least two specific recognition elements capable of specifically attaching and joining at least two surfaces;
- multivalent heteropolymeric hybrid structures which comprise a first synthetic heteropolymer hybridizably linked to a second synthetic heteropolymer;
- aptameric multimolecular devices which comprise a nonaptameric specific recognition pair and a synthetic aptamer which specifically binds or shape-specifically recognizes an aptamer target wherein a member of the nonaptameric specific recognition pair is conjugated to the aptamer to form a conjugated aptamer;
- tethered specific recognition devices which comprise a molecular scaffold and at least two members of a specific binding pair or shape-specific recognition pair.
- tethered specific recognition devices which comprise a molecular scaffold and at least four members of two specific recognition pairs;
- paired specific recognition devices which comprise a molecular scaffold and at least two different specific recognition pairs conjugated to the molecular scaffold.
- a nonaptameric multimolecular device which comprises a conjugated defined sequence segment and at least two different specific binding pairs or shape-specific recognition pairs;
- multimolecular drug delivery systems which comprise a multimolecular structure selected from a group consisting of aptameric multimolecular devices, heteropolymeric discrete structures, multivalent heteropolymeric hybrid structures, synthetic heteropolymers, tethered specific recognition devices, paired specific recognition devices, nonaptameric multimolecular devices, multivalent imprints, and immobilized multimolecular delivery systems wherein the multimolecular structure contains a synthetic receptor that specifically recognizes a drug or a selected target;
- immobilized multimolecular structures which comprise a solid support and a multimolecular structure immobilized to the solid support wherein the multimolecular structure is selected from the group consisting of aptameric multimolecular devices, heteropolymeric discrete structures, multivalent heteropolymeric hybrid structures, synthetic heteropolymers, tethered specific recognition devices, paired specific recognition devices, nonaptameric multimolecular devices, multivalent molecular structures, multivalent imprints, and multimolecular drug delivery systems;
- a shape-specific probes which comprise a nucleotide-based or nonnucleotide recognition element capable of recognizing a specifically attractive surface feature;
- a paired nucleotide-nonnucleotide mapping libraries which comprise a plurality of selected specific recognition partners capable of transposing a selected population of selected nonoligonucleotide molecules into replicatable nucleotide sequences;
- methods for selecting a single synthetic nucleotide molecule capable of recognizing a selected target molecule comprising detecting a signal resulting from the proximity or functional coupling between the single synthetic nucleotide and the selected target molecule. In this method, it is preferred that the single synthetic nucleotide be

selected from a nucleotide library; and
- methods for identifying a specifically attractive surface feature which comprises contacting a surface library with a selected shape-specific recognition partner and detecting attachment of the selected shape-specific recognition partner to a specifically attractive surface feature of the surface library. In a preferred embodiment, the shape-specific recognition partner is detectably labeled and attachment is detected by single-molecule detection.

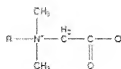
There is nothing in Cubicciotti that would suggest to a person skilled in the art that glycine betaine would be amenable to any of the foregoing molecular machines. In addition, based on the information and materials provided in Cubicciotti, there is nothing to suggest that a person skilled in the art would be able to deduce or derive a slow release dosage form of glycine betaine, as claimed. And even if glycine betaine could be envisioned, a person skilled in the art at the time of the invention would not be motivated to use it in the molecular machines disclosed in Cubicciotti. Accordingly, the teachings of Cubicciotti cannot be said to support or explain the surprising and unexpected results achieved in present application.

MALAMUD ET AL. (U.S. Patent No. 5,928,195)

6. The invention disclosed in Malamud is directed towards the delivery of microbicide drugs comprising a surfactant and an oxide compound. The disclosed drugs use surfactants with spermicidal, antiviral, antibacterial, and antifungal activities, such as a class of compounds comprising an alkyl-N-betaine surfactant in combination with an oxide selected from the group consisting of alkyl-N, N-dimethyl amine oxide, N-dihydroxyethylamine oxide, acylamino t-amine oxide, and mixtures thereof. The drug's activity is centered on the association of the surfactant with the oxide to form a stable micellar structure in the compound.

Again, a person skilled in the art at the time of the present application would not find the claimed glycine betaines to be obvious or predictable in view of the teachings of Malamud. In

particular, the structural, chemical, and physio-chemical properties of glycine betaine do not correspond to the surfactants based on alkyl-N-betaine disclosed in Malamud. For example, as shown in the structure below, alkyl-N-betaine surfactants contain an alkyl chain, which is responsible for generating the surfactant properties with the associated spermicidal, antiviral, antibacterial, and antifungal activities:



Alkyl-N-betaine

where R is a higher alkyl chain generally having at least 10 or more carbon atoms. In contrast, glycine betaine does not exhibit microbicidal properties. This makes sense because, unlike the surfactants disclosed in Malamud, there is no alkyl chain in glycine betaine to generate the associated spermicidal, antiviral, antibacterial, and antifungal activities. Because of these structural and chemical differences, the disclosed alkyl-N-betaine surfactant would also not be expected to generate the therapeutic properties of the claimed glycine betaine to treat thrombosis.

Based upon the teachings of Malamud, it also would not have been obvious to a person skilled in the art at the time of the invention to modify the teachings of Malamud to use glycine betaine. That is, there would also be no scientific rationale to modify the surfactants disclosed in Malamud to remove the alkyl chain and replace it with a methyl group to arrive at the glycine betaine structure. As noted above, Malamud is concerned with microbicide drugs. Removing the alkyl chain would defeat the spermicidal, antiviral, antibacterial, and antifungal activities of the surfactant and render the drug unsuitable for the intended microbicide purposes disclosed in Malamud. This

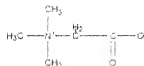
modification would also interfere with the surfactant's interaction with the oxide and inhibit the formation of the micellar structure necessary to create a stable microbicide compound.

Accordingly, the teachings of Malamud cannot be said to support or explain the surprising and unexpected results achieved in the present invention.

MURPHY ET AL. (U.S. Patent No. 6,355,166)

7. The invention of Murphy is directed towards the optimization of new substituted fullerenes which are completely different chemical structures compared to the present glycine betaines. These fullerene derivatives are based on carbon atoms forming nanostructures such as hollow spheres, ellipsoids, and tubes.

Murphy discloses different approaches to functionalize these fullerenes. Among the disclosed approaches, Murphy makes reference to reacting the fullerenes with glycine derivatives, such as N-aryl glycine, in order to produce R-substituted fullerenes. However, as can be seen from the Murphy reference itself (for example in column 24, lines 10-40), the intermediate and final R-substituted fullerene products are still totally different from glycine betaine:



Glycine Betaine



R-substituted Fullerene


A person of ordinary skill in the art would also not find the claimed glycine betaines to be obvious or predictable based upon the teachings of Murphy. In particular, the properties of the fullerene carbon cages are well known in the art, and the disclosed substituted fullerenes have

properties totally different from glycine betaine in terms of chemical structure (as can be seen above), molecular weight, and solubility. There is also no disclosure in Murphy at all regarding betaines. Therefore, a person skilled in the art would not be motivated to modify the compounds disclosed in Murphy to arrive at the claimed glycine betaine.

Based upon my extensive experience in drug delivery systems, a person skilled in the art at the time of the invention would not be able to deduce or to derive a slow release dosage form of glycine betaine, as claimed, based on the information and materials provided in Murphy. There is also no scientific rationale in Cubicciotti, Murphy, or Malamud, taken individually or combined, which could explain or provide a reasonable expectation of success in achieving the surprising and unexpected results of the invention claimed in the present application, based upon the information provided in these references.

8. I further declare that all statements made herein are true within the limits of my own knowledge and all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that wilful, false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and such wilful false statements may jeopardize the validity of any patents issued from the patent application.

Date:

11/02/2008
November 2, 2008

Dr. Christian Grandfils

1. Personal data

Professional address : Interfaculty Center for Biomaterials (CEIB) ; www.ulg.ac.be/ccib
Chemistry Institute, B6c, 4000 Liège (Sart-Tilman), Belgium (phone : 32(0)43663416/3506 -
e-mail : C.Grandfils@ulg.ac.be Mobile phone : 32 (0)496 185981

Position :

- Assistant Professor : University of Liège, medicine faculty.
- Director of the Interfaculty Biomaterial Centre (CEIB) ULg
- Scientific consultant in the field of biomaterials for medical industries and polymers for pharmaceutical companies.

2. Scientific Background

- Ph.D. in Biomedical and Experimental Sciences, University of Liège, 1988, in the Laboratory of Professor E. Schoffeniels, General Biochemistry.
- Post-Ph.D. research activities in the field of the polymers designed for biomaterial applications.

3. Research fields

- Tissue engineering for bone reconstruction : optimisation of biocompatible, biodegradable scaffolds, hydrogels.
- Drug delivery systems : encapsulation of biopharmaceutical compounds (peptides, proteins, DNA) considering different vectors : micelles, nanoparticles ; microparticles, hydrogels.
- Optimisation of diagnostic systems : physical immobilisation of enzyme within polymer matrices.
- In vitro biocompatibility testing of biomaterials (hemocompatibility ; cell cytotoxicity)

5. Member of Scientific Societies

- Belgium Pharmaceutical Society, since 1999.
- Belgium Microscopy Society, since 1999, and member of the board of this Society and treasurer, 2001.
- Belgian Branch of the European Society for Animal Cell Culture Technology" BELACT, since 2000, and member of the board of this Society, 2001.
- Biolîège (Wallonne Association of Biotechnologists), member of the « board » since 2001), and secretary since 2004.

6. Publications/ Patents

Book chapters : 8 ; International patents : 6 ; International publications : 82 ; Publications in national journals : 8 ; Communications/abstracts of conferences : 90.

7. Collaborations with industries

Belgium : Physioli, Eurogentec, SGS Lab. Simon, Kitotyzme, SEGAL, Zentech, Henogen, UCB Pharma, Baxter, GSK, InBev **France** : Coletica, Euraceli ; **Germany** : Celonic, Glatt International, genialLab, AcrossBarrier ; **Switzerland** : Debiopharm Galenic, Debiopharm, AMCIS, Meiners Commodity Consultant S.A., Sochinaz, **Netherlands** : Medtronic.

